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MONTCLAIR STATE UNIVERSITY

Learning and phototaxis in *Belostoma flumineum*

By

Kaitlin Garvey

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

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## Abstract

Learning allows organisms to adjust their behavior to adapt to a changing environment. The premise of this study was to investigate the capacity for learning in the giant waterbug, *Belostoma flumineum* Say (Heteroptera: Belostomatidae). As part of these experiments, I was able to identify an apparent directional phototaxis and to use it as motivation in an operant conditioning study. *Belostoma flumineum* has been observed to exhibit negative directional phototaxis. The first two parts of this study suggest that *B. flumineum* has a statistically significant preference for shadowed over illuminated areas, as well as a preference for black substrate over white substrate when introduced into a basin. These preferences are most likely linked to their life history. *Belostoma flumineum* typically hides in darker or shaded areas to avoid predators and stealth capture prey. These abilities and preferences were used in a subsequent experiment to investigate potential adaptive learning in *B. flumineum*. Given the predictable preference of *B. flumineum* for shaded areas, I was able use shade as a basis for a reward system. The waterbugs were rewarded with darkness for choosing the designated behavior. The results suggested that the waterbugs learned to associate a white substrate with the reward. During the experiment, the waterbugs appeared to have a bias toward one side of the room when introduced into the basin, but this did not statistically account for the association between positional behavior and reward. This study provides insight into the strength of directional phototaxis and adaptive learning in *B. flumineum*.

LEARNING AND PHOTOTAXIS IN *BELOSTOMA FLUMINEUM*

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Biology

by

KAITLIN ANN GARVEY

Montclair State University

Montclair NJ

2014



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## Introduction

Learning is the exhibition of a change in the behavior of an organism as a result of a particular experience (Thorpe, 1943; Thorpe, 1963; Punzo, 1984; Kazdin, 2001; Dukas, 2008). If an appropriate behavioral pattern is learned, it can increase the fitness of an organism and potentially increase its fecundity (Padaj and Lewis, 1993; Dukas, 2008). Learning is presumably an approach for adapting to environmental instability and change. For natural selection to favor learning, the acquisition of information must benefit the organism in some way, and this benefit must outweigh the costs of learning it (Dukas, 2008). Motivation is one of the driving forces behind the ability for an organism to learn, for without the prospect of a future reward for a change in behavior, learning may not occur (Dukas, 2008). Some adaptations associated with learning include feeding, reproduction, predator avoidance, aggression, social interactions and sexual behaviors (Dukas, 2008).

Two studies on members of the order Lepidoptera (*Battus philenor*, and *Agraulis vanilla*) examined the ability of butterflies to associate a color with a reward (Weiss, 1995; 1997). Weiss concluded that the butterflies could associate different colors with a nectar reward. The butterflies quickly learned which color to associate with nectar. Even though both subjects in the studies innately preferred yellow flowers, they learned quickly to go to flowers of different colors when the yellow ones did not have any nectar (Weiss, 1995; 1997).

Learning is also a part of avoidance behaviors. Lee and Bernays studied the effect of food aversion in a polyphagous grasshopper, *Schistocerca americana* (1990). They tested to see if *S. americana* could associate the toxic effect of different plant compounds

with the taste of new foods. When the grasshoppers were given a new food, and when they had finished eating it, they were injected with a toxin. After the grasshoppers had felt the effects of the toxin, they no longer ate the new food (Lee and Bernays, 1990). Lee and Bernays suggest that *S. americana* made the association between the toxin and the food (1990).

Many invertebrates exhibit some kind of directional phototaxis (Welsh, 1934; Kelly and Mote, 1990; Zhang, 1992; Staddon, 2003; Orlosk *et al.*, 2011). Phototaxis is the ability to differentiate between light and dark and orient in the appropriate direction (Staddon, 2003). A study involving mite larvae, *Allothrombium pulvinum*, investigated their directional phototaxis (Zhang, 1992). When placed into a tube that was illuminated on one side and shaded on the other, more mites moved to the illuminated end. This study suggests that *A. pulvinum* are positively phototactic (Zhang, 1992). Directional phototaxis has also been suggested in species of cockroach, crayfish and crabs (Welsh, 1934; Kelly and Mote, 1990; Zhang, 1992; Staddon, 2003; Orlosk *et al.*, 2011).

*Carcinus maenas* is a species of crab whose behavior suggests that the crab instinctively has a negative directional phototaxis (Orlosk *et al.*, 2011). Orlosk *et al.*, conducted a learning experiment using the negative directional phototaxis of *C. maenas* (2011). The objective of the experiment was to determine whether *C. maenas* could make an association between an illuminated area and a reward. The experiment consisted of a series of training trials culminating in a learning association trial. The crabs were placed on a large grid and a light was projected on one of the grid squares; if the crabs moved to that square they received reward of food. Orlosk *et al.* asserted that for the crabs to make

the association between the lit square and the food reward they had to act against their natural light avoidance instincts (2011).

Learning involves neuronal modifications in the brain, and it can only be measured indirectly through the behavior of an organism (Dukas, 2008). Behavioral changes are usually qualitative and not quantitative. The presence or absence of a behavior in a particular trial does not necessarily indicate that there was no previous neuronal modification in the brain; it may reflect low motivation or problems with the methodology of the study. Therefore, a control group is always necessary to eliminate some of the external circumstances that may affect the study (Dukas, 2008).

Learning studies can be complicated, because they require that organisms be provided with the proper motivation to change their behavior and perform a new task. The present study is based on the principle of operant conditioning, or trial and error learning. Operant behavior is a behavior that is guided by consequences (Kazdin, 2001; Brembs, 2003; Staddon, 2003; Cammaerts, 2004; Wade and Tavis, 2005). In operant conditioning, a chosen behavioral response is modified using a reward or punishment.

Operant conditioning was used in a study of the ant *Myrmica sabuleti*, to encourage the ants to make an association between a visual cue and a food reward (Cammaerts, 2004). Cammaerts set up an apparatus near an ant colony that contained either sugar or meat; the ants took food from it and brought back to their colony (2004). After several days, the apparatus was removed and an identical one, with no food in it, was placed in a new location. Cammaerts then counted the number of ants that went to the new apparatus. The study concluded that *M. sabuleti* associated the visual cue of the apparatus with food (Cammaerts, 2004).

Adaptive learning is modification of a behavior that results in increased fitness and reproductive success (Egas and Sabelis, 2001). An adaptive learning study on an herbivorous mite, *Tetranychus urticae*, evaluated the importance of host preference and its effect on overall fitness (Egas and Sabelis, 2001). Two separate strains of *T. urticae* were initially given a choice of either tomato or cucumber plants to eat and lay their eggs on. The experiment suggested that the first choice of host plant by a mite affected its second and third choices. The cucumber plant allowed for increased oviposition, survival and development. The mites that chose the cucumber plant first remained with the cucumber plant for the second and third trials. A significant portion of the mites that chose the tomato plant on their first trial switched over to the cucumber plant on the second trial and went back to the cucumber plant again for the third trial (Egas and Sabelis, 2001). The cucumber plant served as a better host and Egas and Sabelis propose that the mites that chose the cucumber plant would have a higher fitness (2001). This experiment suggests that *T. urticae* increased their reproductive fitness as a result of behavioral changes that are associated with previous experiences (Egas and Sabelis, 2001).

There have only been a few studies on adaptive learning in Heteroptera (Hénaut *et al.*, 1999; 2000). As a basis for this experiment, I investigated the possibility of eliciting a learning response from a giant waterbug. I chose a member of this group because I have previously observed that they have a strong preference for darker areas, which would provide a suitable reward for a conditioning experiment.

The family Belostomatidae is a member of the insect order Heteroptera and the infraorder Nepomorpha (Hebsgaard *et al.*, 2004). The insects in this family are

commonly known as giant waterbugs, and the organism analyzed in this particular study was *Belostoma flumineum* Say (Daly *et al.*, 1998; Hebsgaard *et al.*, 2004). *Belostoma flumineum* ranges in size from 18 to 24 mm and can be found in small freshwater ponds across the United States (Smith, 1976). Members of the subfamily Belostomatinae all exhibit male back brooding (Smith, 1976; Kight and Kruse, 1992; Kruse, 1990; Schuh and Slater, 1995; Daly *et al.*, 1998; Tallamy, 2001). During mating and copulation, the female deposits the eggs on the dorsum of the male. The male provides exclusive post-copulatory care of the eggs until they hatch, which usually takes place 6 to 12 days later (Kruse, 1990; Kight and Kruse, 1992). Encumbered males are typically found near the surface in emergent vegetation, while females and unencumbered males are found near the bottom of the pond under mats of vegetation (Smith, 1976).

Giant waterbugs appear to select habitat based on their mode of feeding. They hide and wait in dark areas for the arrival of prey species, which typically consist of small invertebrates, and then they attack (Severin and Severin, 1911; Schuh and Slater, 1995; Daly *et al.*, 1998). They capture their prey and inject them with a powerful saliva made up of toxins and proteolytic enzymes that liquefy the tissues of the target, which are then ingested through piercing sucking mouthparts (Severin and Severin, 1911; Polhemus and Polhemus, 2008). Presumably, *B. flumineum* also exhibit preferences for dark areas to avoid predators like *Dolomedes triton*, fishing spiders (Kraus, 1989).

The present study makes the prediction that *B. flumineum* exhibits a negative directional phototaxis, preferring shadowed, rather than lighted areas. The premise of this study was to investigate directional phototaxis in *B. flumineum* as well as their capacity for learning and making associations between visual stimuli. This study uses the theory of



adaptive learning to investigate whether waterbugs have evolved to make associations about important cues like dark versus illuminated habitat.

## Methods

Giant waterbugs, *Belostoma flumineum* Say, were collected from August to November 2012 from several freshwater ponds and small lakes in Sussex County, New Jersey. I collected adult specimens from the perimeter of the lakes using aquatic dip nets. The animals were then transported to the laboratory at Montclair State University in large coolers filled with water. The waterbugs were immediately sexed, and the males and females were sorted into different tanks. The waterbugs were stored in a temperature-controlled environmental chamber at 26° C. They were fed crickets every 2 to 3 days and debris was removed from the tanks following feeding bouts. The tanks were cleaned and the water was changed every two to three weeks.

The experiments were initiated in January 2013. All experiments were conducted in an arena constructed from a large plastic basin (44.5 cm diameter) filled with water to a depth of 5.1 cm. To account for potential behavioral differences between sexes, only female *B. flumineum* were used in this study. The experiment was conducted in the laboratory at room temperature, approximately 23°C. The basin was marked around the perimeter with the numbers 1 through 12 in clock positions (Figure 1). In each experiment, a subject was released with its head facing downward from a height of 25.4 cm above the water surface into the basin. The orientation of the waterbugs relative to the arena was randomized for each waterbug introduction, so that when each waterbug fell, it faced a random side of the basin. Because subjects were repeatedly introduced into the basin, each waterbug was left undisturbed for approximately one minute between trials to minimize stress. All of the waterbugs were marked with different color nail polishes on their pronotum for identification. Additionally, there were not enough individuals to

allow for all independent trials, so some of the waterbugs were reused in multiple experiments.

*Experiment 1: Do B. flumineum exhibit phototaxis?*

The first experiment tested whether or not *B. flumineum* exhibited a preference for shaded areas in the arena. In preliminary trials, prior to Experiment 1, waterbugs were introduced in the far illuminated side of the basin and swam across to the shadowed area. In response to these initial informal observations about the behavior of the waterbugs, I sought to quantify the information. The intention of this experiment was to provide a quantitative estimate for preference of the waterbugs for shadowed areas. An opaque cardboard barrier was placed perpendicular to the surface of the water, and a 60 watt incandescent clamp light (22.7 cm diameter, 800 lumens) was placed over one side of the basin, 17.9 cm above the water surface, so that the opposing side was shaded (Figure 2). The lights were shut off in the room and each waterbug was introduced into the basin 10 times, when the waterbug stopped swimming, this was usually within 30 seconds, its final location was noted as either the dark side or the light side of the arena (n=34). The waterbugs were given a 1-minute undisturbed period in between each introduction.

*Experiment 2: Are B. flumineum attracted to black or white backgrounds?*

The second experiment tested whether the subjects reacted to a visual stimulus, and additionally acted as a control trial for Experiment 3. In this experiment, rather than shading half of the basin as in Experiment 1, I covered half of the walls with white paper and the other half with black (Figure 3). This approach tested whether or not the

waterbugs had a bias toward black, as opposed to white, visual cues. Two 6.4 cm x 4.5 cm strips of black paper were covered in contact paper and placed around the edge of the basin. The process was then repeated with two 6.4 cm x 4.5 cm strips of white paper, so that half of the wall was black and the other half was white (Figure 3). The same light used in Experiment 1 was placed directly over the basin to illuminate the entire arena. The waterbugs were placed in the arena using the same method of introduction in Experiment 1. Each waterbug was introduced into the basin 20 times and when the waterbug stopped swimming, its location was noted (n=39). The water bugs were given a 1-minute rest period in between each introduction.

I suspected that additional trials would be necessary, because preliminary observations suggested a weak response to the stimulus of black and white paper around the basin. I noticed in preliminary observations that the waterbugs seemed to have a preference for the southwest corner of the basin, regardless of its color. To account for this potential preference, I rotated the basin after every 5 introductions, and I noted whether the preferred corner was black or white. When subjects stopped swimming and came to rest, their location was noted based on the wall color of the side that they were on, either on the side with the black wall or on the side with the white wall.

### *Experiment 3: Do *B. flumineum* learn to associate a visual cue with a reward?*

The final experimental treatment used operant conditioning to test whether waterbugs could learn to associate the white wall with the putative reward of darkness. Experiments 1 and 2 suggested that the waterbugs are negatively phototactic and that

they had a preference for a black wall (see Results). Darkness was therefore used as a positive reinforcer in this experiment.

The waterbugs were introduced into the arena as in the previous experiments, and, similar to Experiment 2, half of the basin wall was covered in white paper, while the other half was covered in black and the basin was rotated every 5 bug introductions. Like the other experiments, a clamp light was placed above the basin. In this experiment, the lights in the room remained off, and the light above the basin was the only light source, so that when the light was extinguished, the interior of the arena would be darkened. Experiment 3 was subdivided into two stages: Stage 1, a trial in which the waterbugs were rewarded for a partial response, followed by Stage 2, a trial in which the subjects only received a reward for a full response.

The purpose of Stage 1 was to provide the animals with the opportunity to make an association between the white half of the basin and the reward. The waterbugs were each introduced into the basin 20 times using the same method as in Experiment 2. When subjects swam toward the white half of the basin, they were rewarded with darkness as the overhead clamp light was shut off, regardless of whether or not they remained on that side. The light remained off for one minute, and then the waterbug was removed from the basin. If the waterbug did not swim into the white side of the basin and stopped on the side of the basin with black walls, then no reward was given and the waterbug was removed from the basin. In both cases, I noted what side of the basin the waterbug chose.

Stage 2 of this experiment was an extension of Stage 1. The waterbugs were left undisturbed for 30-minutes after Stage 1, in an effort to minimize stress. In Stage 2, the waterbugs were only rewarded with darkness if they stopped on the side of the basin with

the white wall. If the waterbug stopped on the side of the basin with the white wall, the lights were turned off for one minute, and the waterbug choice recorded. If the waterbug stopped on the side of the basin with the black wall, the waterbug was removed, and I noted that the waterbug chose the black side of the basin. The apparatus was identical to Stage 1, but the number of repetitions was increased to 30 to increase resolution of any associations that waterbugs exhibit, without excessively increasing stress levels.

## Results

The statistical results described below were completed using the statistical software package R (R Core Team, 2013; Kern, 2013). R is an open source statistical application capable of running the various statistical models described below.

### *Experiment 1: Phototaxis*

Experiment 1 investigated whether *B. flumineum* exhibits phototaxis. The data suggest that probability that the waterbugs (n=34) will go to the illuminated side of the basin was 6.8% (lower confidence limit: 4.3%; upper confidence limit: 10.4%) (Figure 4). However, these results may have been confounded because the basin was not rotated and the southwest positional bias was not accounted for. The southwest corner of the basin was black for all of the trials. Despite the preference related to the orientation of the basin within the room, the waterbugs still appeared to exhibit a very strong preference for the shadowed side of the basin. The preference of waterbugs for shaded areas was also evident in the later experiments.

### *Experiment 2: Black and White Preference*

The purpose of Experiment 2 was to quantify the behavior of the waterbugs prior to operant conditioning. Given the preference for shade evident in Experiment 1, I suspected that subjects might show a preference for black over white visual cues. This preference was expressed as a probability for selecting the white substrate. In addition to the preference of the waterbugs for the black side of the basin, the statistics also indicated that there was a room orientation bias centered on the southwest corner of the basin.

Thirty-nine waterbugs were tested for this experiment with 20 introductions per waterbug ( $n=39$ ). When the southwest corner was black, the waterbugs chose the white side of the basin only 25% of the time. When the southwest corner was white, the waterbugs chose the white side of the basin 31% of the time (Figure 5). If the room orientation bias was ignored *i.e.*, if the results of these two orientations are simply averaged, the waterbugs would have chosen the white side of the basin 28% of the time. The statistical uncertainties of these numbers are derived later in this section.

### *Experiment 3: Learning*

Experiment 3 was conducted to see whether waterbugs change visual cue preferences in response to operant conditioning. The data from Stage 1 of Experiment 3 are not a part of the analysis, because the treatment was intended to allow the bugs to begin to make the association between the stimulus and the reward, rather than to yield quantitative data. Twenty-one waterbugs were tested for this experiment with 30 introductions per waterbug ( $n=21$ ). When the southwest corner was black, the waterbugs chose the white side of the basin and remained there only 35% of the time. When the southwest corner was white, the waterbugs chose the white side of the basin 41% of the time (Figure 6). If the room orientation bias was ignored *i.e.*, if the results of these two orientations are simply averaged, the waterbugs would have chosen the white side of the basin 38% of the time.



*Assessment of uncertainty and quantitation of the effects of the individual variables*

The primary statistical analysis used to assess the uncertainties on the four proportions in Experiments 2 and 3 was the beta binomial distribution. This was necessary because the individual experiments represent binomial variables. The result of each bug introduction was measured as a success or failure *i.e.*, a binomial outcome (Box *et al.*, 1978; Berthouex, 1994; Moore *et al.*, 2012). In my experiment, the bug going to the white side of the basin was considered a success and the bug going to the black side of the basin was considered a failure. The results of multiple bugs each being introduced multiple times will describe a binomial distribution (normal distribution experiments are not measured this way) (Box *et al.*, 1978; Berthouex, 1994; Moore *et al.*, 2012). Normal variables yield continuous outcomes, while binomial variables can only yield two. The graph of a beta binomial distribution looks very similar to the graph of a normal distribution, in that it has the same bell-shaped curve, but the confidence limits are calculated differently.

In a binomial distribution, all trials are independent, and the probability of a success or failure is the same for each (Berthouex, 1994; Box *et al.*, 1978; Moore *et al.*, 2012). A simple binomial distribution could be used in flipping coins. Assuming that all of the coins used are fair, all of the trials are independent, and the probability of success does not change. This is not the case with the bugs, however; while the results are considered binomial, all of the bugs are not the same, and probability of success may not be the same for every trial. A beta binomial test is used instead, because it accounts for the variation between subjects and trials (Lord, 1965; Chatfield and Goodhardt, 1970; Williams, 1975; Wilcox, 1981; Madden *et al.*, 1995). A beta binomial distribution was

used in a study by D. A. Williams on animal litters (1975). Williams used the beta binomial distribution to calculate the variation between fetuses in the same litter, as well as between litters, with the same treatment. I used the same idea in doing the analysis for this experiment. I used the beta binomial distribution to calculate the variation between bug introductions for each bug, as well as between bugs.

The probabilities mentioned above can be calculated without the beta binomial analysis; this analysis calculates the distributions or confidence limits associated with the data (Lord, 1965; Chatfield and Goodhardt, 1970; Williams, 1975; Wilcox, 1981). The beta binomial distribution accounts for the binomality of the data and gives the uncertainty for individual probability.

A beta binomial regression analysis was performed on the data from Experiments 2 and 3. A regression estimates the relationship among the variables and finds the best coefficients to express the relationship with the minimum amount of variance in the dependent variable (Moore *et al.*, 2012). The regression analysis uses a maximum likelihood method to estimate the uncertainties in the data. It takes maximum advantage of the data to minimize the uncertainty effect (Griffiths, 1973; Crowder, 1978).

The objective of this analysis was to separate the learning and room orientation variables, while at the same time estimating the uncertainties associated with the measured probabilities. Ideally, it would be better to compare the control and learning trials directly, but the confounding variable, the room orientation for the southwest quadrant of the arena, prevented this. The regression of the data is a way to optimize the data, even though it is confounded by the room orientation bias parameter (Moore *et al.*, 2012). The probability that the bugs go to the white side is a function of trial type

(control, training) and the room orientation bias. The beta binomial regression gives the relationship between the room orientation bias and the learning parameters in the form of an equation with coefficients; it is defined by the following formula:

(1) Beta Binomial Regression Model

$$\ln\left(\frac{p}{1-p}\right) = c + b(RB) + a(L)$$

$$\ln\left(\frac{p}{1-p}\right) = -1.108 + (0.2897)(RB) + (0.4516)(L)$$

where:

$p$  = Probability that the event will occur

$RB$  = Room orientation variable (If the biased corner was black it is 0 and if the biased corner was white it was 1)

$L$  = Learning variable coefficient (The waterbugs in the control trial (Experiment 2) were given a 0 and the ones in the learning trial (Experiment 3) were given a 1)

$c$  = y-intercept

$b$  = Coefficient associated with the room orientation bias

$a$  = Coefficient associated with the learning trial

Under this regression, the coefficient for the room orientation bias was 0.2897 with a p-value of 0.0305. This observation provides further evidence to suggesting the preference of the waterbugs for one corner of the basin, regardless of its color and the trial. Even though this value is statistically significant, it still has a relatively weak effect. The same regression indicated that the coefficient for the learning variable was 0.4516

with a p-value of  $8.05 \times 10^{-4}$ . These results suggest that the learning effect had more influence on the behavior of the waterbugs than the orientation of the basin because the learning effect has a larger coefficient. A greater coefficient indicates a stronger response (Moore *et al.*, 2012).

The model above was used to generate the 95% confidence limits for the probabilities mentioned above (Tables 1 and 2, Figure 7). Figure 7 is a graphical representation of the data, which compares the preference of the waterbugs for the white side of the basin under the four different conditions described in Table 2. Figure 7 and Table 1 also include the lower and upper confidence limits as calculated by the beta binomial distributions. The graph indicates three things, the first being that the bugs had a preference for the black backgrounds in all conditions *i.e.*, all probabilities for selecting the white side were less than 0.5. Secondly, there was a room orientation bias; the color of the biased corner did have an effect on where the bugs chose to go. The probability of a waterbug choosing the white side of the basin was higher when the southwest corner was white, and the probability of a waterbug choosing the white side of the basin was lessened when the black side when the southwest corner was black. Lastly, the learning trials had an effect on the waterbugs; there is a difference between the control trials and the learning trials.

The final calculation done was the odds ratio. The odds ratio provides an estimate for the link between two variables with binomial results, and they provide a way to represent a probability (Bland and Altman, 2000). The odds ratio represents the degree to which the learning trials have increased the odds that a waterbug will choose the white

side, after adjusting for variation in the data due to the location on the biased corner (Bland and Altman, 2000). The odds ratio is defined by the following equation:

(2) Odds ratio

$$\text{Odds Ratio} = e^{(a)}$$

$$\text{Odds Ratio} = e^{(0.4616)}$$

If the odds ratio is 1, then there is no difference between the control trials and learning trials. However, if the odds ratio is significantly greater than 1, then there is a difference (Bland and Altman, 2000). According to my model, the odds ratio is 1.57 (lower confidence limit: 1.21 and upper confidence limit: 2.05). Despite the room orientation bias effect, the bugs in the learning trials preferred the white side more than the bugs in the control trials. The probability of the waterbugs choosing the white side of the basin increased by 57 percent after the operant conditioning. The p-value associated with the learning coefficient above ( $p = 8.05 \times 10^{-4}$ ) is also associated with the odds ratio, because the learning coefficient is used to calculate the odds ratio. (See appendix for explanation of covariance between learning and room orientation bias.)

## Discussion

### *Phototaxis*

This study suggests the capacity for learning in *B. flumineum*, through use of their strong directional phototaxis. In all three experiments, *B. flumineum* exhibited directional phototaxis. Giant waterbugs behaviorally discriminate between shadowed and illuminated areas, as well as black and white substrates. Over all, they exhibited a preference for darker areas. Discrimination between degrees of ambient light or substrate darkness could be important because giant waterbugs are typically sit-and-wait predators that conceal themselves in dark areas and wait for their prey to come to them (Severin and Severin, 1911; Schuh and Slater, 1995). Preference for dark areas in *B. flumineum* could also aid in crypsis. Adult giant waterbugs are dark brown in color and would blend in more effectively with a black background than a less pigmented one. An experiment by Kettlewell and Conn investigated crypsis in some moths in the order Lepidoptera (1977). The moths chose to perch on areas where they were most camouflaged. It is possible that when the giant waterbugs chose the black side of the basin, it was an attempt to conceal themselves. The preferences of *B. flumineum* relate to their need to stay hidden so that they can catch prey and avoid predators

### *Learning*

Learning occurs when the present behavior of an organism is changed by a past experience (Thorpe, 1943; Punzo, 1984; Egas and Sabelis, 2001). Natural selection presumably favors individuals that are best at learning associations that lead to increased survival and reproduction (Smith, 1993; Staddon, 2008). If an organism can make an

association between stimuli in its environment, it may be able to use this association to increase its fitness and fecundity (Smith, 1993; Honda and Kainoh, 1998; Egas and Sabelis, 2001; Staddon, 2008).

Capacity for learning can affect fecundity. A study by Honda and Kainoh investigated the effect of learning on fecundity in *Ascogaster reticulatus*, an egg-larval Hymenoptera parasitoid of smaller tea tortrix (1998). In their experiment, *A. reticulatus* associated tea leaf extract, usually found in the diet of the smaller tea tortrix, with a host to deposit their eggs. The females in this study did not have an innate preference tea leaf extract (Honda and Kainoh, 1998). The ability of *A. reticulatus* to make these associations is related to their oviposition rates. The study suggests that females able to make the association laid more eggs and had higher fecundity (Honda and Kainoh, 1998).

Learning new behaviors is presumably adaptive when the benefits of learning that behavior offset the costs *i.e.*, time and energy expenditure (Dukas, 2008). Time must be invested in order to acquire a new behavior, and this is especially important in the case of insects, because they have relatively short life spans. Energy is also invested when making new behavioral associations; there is a cost involved in the processing, storing and maintenance of the neuronal structures related to memory (Mery and Kawecki, 2003; Dukas, 2008). Too much time and energy spent learning a new association can decrease the lifespan of an insect and possibly reduce its fecundity (Mery and Kawecki 2003; 2004; Snell-Rood *et al.*, 2011).

Mery and Kawecki identified the operating costs for learning in their studies on fruit flies, *Drosophila melanogaster* (2003; 2004). Two strains of fruit flies were bred, one with higher learning potential and one with lower learning potential. The fruit flies



that were bred to be high learning had a shorter life span and had lower egg laying rates (Mery and Kawecki, 2004). Another study involving larval competitive ability suggested the same idea. The flies that were considered to have lower learning abilities were more competitive (Mery and Kawecki, 2003). Mery and Kawecki speculated that these differences were due to the energy spent in collecting, processing and storing information.

These studies suggest that learning can be maladaptive and that natural selection might select against learning ability. This may be true to a certain extent, in that there is a limit to time and energy spent in learning and that too much time might also reduce fecundity. These studies on *D. melanogaster* suggest that too much time learning can have a detrimental effect on the fitness of the organism. However the subjects were selected in the lab to be higher learning, and in the natural habitat of *D. melanogaster* these more extreme learning abilities may not exist. Natural selection produces populations of organisms that balance the cost of learning with the benefits of a new ability or association. Learning has a high cost to the organism, and it is only likely to be favored by natural selection if the acquired behavior will benefit to the organism in the future (Dukas, 2008).

Learning can be viewed as the process of an organism reacting to uncertainty in its environment, whether the environment is natural or artificial (Smith, 1993). Natural selection favors learning in an environment that is slightly unpredictable, where the learned information has some effect on fitness (Alcock, 2005). An environment that changes slowly over time is more ideal for learning. It would give the organisms the time to make new associations and to use them. In extremely unpredictable environmental



conditions, learning may not be useful, because important variables change too quickly (Stephens, 1993). If an environment is too unpredictable, that environment may change before the organism can use its new association. Learning has the same cost-benefit structure that is associated with many other biological principles. Learning mechanisms are costly therefore learning should only occur when there is some major effect that outweighs the cost (Alcock, 2005).

All three experiments in the present study indicated that *B. flumineum* prefer dark or black areas. In their natural environment, waterbugs typically exhibit a preference for darker areas, presumably to catch prey and avoid predators. The results indicated that the waterbugs learned to make an association in order to obtain a darkened basin as a reward. This suggests that *B. flumineum* has an adaptive requirement for shadowed areas. Even though it is clear that the waterbugs prefer the black background, they were able to make the association between the white background and the reward.

This study as well as three previously mentioned studies proposes a similar interpretation for results (Weiss 1995; 1997; Orlosk *et al.*, 2011). Studies by Weiss on several members of the order Lepidoptera suggested that the butterflies could act against their natural attraction for yellow flowers and learn to look for nectar in flowers of other color (1995; 1997). Orlosk *et al.*, provided evidence for learning in a species of crab. The study suggests that the crabs were able to act against their innate light avoidance behaviors to obtain a food reward (Orlosk *et al.*, 2011). In all four studies, the subjects learned to behave differently than their presumably instinctive behaviors to obtain a reward. Studies like these recognize the importance of adaptive learning.

The results from Experiment 1 were confounded by room orientation bias, but they still suggest that *B. flumineum* has a very strong preference for shaded areas. This result suggested that removal of light could serve as a reward for Experiment 3. The waterbugs were rewarded with darkness (the lights were temporarily turned off) when they stopped on the white side of the basin. There is a statistically significant difference between the waterbugs in the control trials (Experiment 2) and the waterbugs in the learning trials (Experiment 3) (Table 1). The waterbugs that went through the learning trials were more likely to go toward the white side of the basin. Specifically, the probability of the bugs choosing a light colored substrate increased by 57% upon completion of the learning trials. Not only does this suggest the capacity for learning in giant waterbugs, but it also suggests the salience of shade as a stimulus.

#### *Room Bias*

The existence of a room orientation bias is very interesting. It is unclear why the waterbugs would have a preference for the southwest quadrant of the arena. There may have been an external cue they were responding to. There is also evidence they have a sensory apparatus to detect pressure waves propagated through water. A study on *Abedus indentatus*, (Heteroptera: Belostomatidae), observed that males exhibit a pumping display to attract females and that all giant waterbugs use surface wave communication (Kraus, 1989). *Abedus indentatus* have hair sensillae, which allow them to sense vibrations in the water. Female *A. indentatus* also use their hair sensillae for mate choice (Kraus, 1989). Smith (1979) observed the same pumping behavior in male *B. flumineum*, but there have not been any studies done on how sensitive the waterbug species is to vibrations. Since

female giant waterbugs would detect and respond to slight vibrations caused by males, it is possible that there were extraneous vibrational cues in the building that subjects detected and reacted to.

### *Pseudoreplication*

Pseudoreplication occurs when the replicates in an experiment are not completely statistically independent (Hurlbert, 1984; Heffner *et al.*, 1996). That is, the outcome of one trial is correlated with the outcome of another. A common mistake and example of pseudoreplication is over-counting the degrees of freedom. For example, in Experiment 2, if I assumed that all of the introductions were independent, then this would yield 780 degrees of freedom. However the 780 introductions were conducted using only 39 waterbugs. Thus, each waterbug was introduced 20 times. These 20 introductions are not independent, because they involved the same waterbug, but neither are they completely dependent.

An experiment that does not involve any type of pseudoreplication is difficult to construct. In a pure experiment, everything would be completely independent and randomized. Ideally, for my experiments, I would have had an unlimited supply of randomly selected waterbugs from the entire *B. flumineum* population, and none of the bugs would have been reused for other experiments. However, given the difficulty of obtaining test subjects, this was not possible.

In Experiment 2, a more correct account of the degrees of freedom would be one for each waterbug, or 39 in total. However, this does not account for the information contained in the individual waterbug introductions. The beta binomial distribution

addresses some of the issues with pseudoreplication (Hurlbert, 1984; Heffner *et al.*, 1996). One of the advantages of using the beta binomial test is that it gives 1 degree of freedom for each waterbug, rather than one degree of freedom for each bug introduction, while it is also able to incorporate information contained in the individual introductions. The beta binomial test recognizes the uncertainty for an individual waterbug, and it takes into account the number of trials that were used to get the initial probability. The number of trials, or in this case the number of bug introductions, affects the uncertainties of the probabilities.

#### *Future Studies*

This study encourages additional experiments. This is the first study on learning in giant waterbugs or in any member of the family. Since this study was performed on only females, the next logical step in the analysis of giant waterbug learning would be to do an evaluation of learning in male *B. flumineum*. Male *B. flumineum* brood eggs on their backs, which involves remaining close to the water surface to maintain the proper balance of hydration and aeration of eggs (Smith, 1976; 1997). Males may be more vulnerable when they are brooding their eggs. A mistake might be more costly for an encumbered male and they may have a higher predisposition for learning. They may be able to make associations faster than females.

Another experiment that could follow this is one would be a test wherein the bugs are exposed to the stimuli and the reward over several days to see if results change. Continued exposure over several days could increase the probability that the bugs would choose the white side of the basin. A third study could investigate the extinction of the

behavior. After it has been established that the bugs have made the association, it would be interesting to investigate how long they will continue to perform the behavior if no reward is given.

This study also indicates some of the visual abilities of *B. flumineum*. General observations were made about the ability of waterbug to discern shadow from light, as well as their ability to discern and have a preference for a black substrate over a white one. These visual capabilities suggest many other future studies regarding color differentiation, pattern differentiation and maze training (Vowels, 1965; Weiss 1995; 1997).

### *Conclusion*

This study provides evidence for adaptive learning in *B. flumineum*. After identifying an apparent negative directional phototaxis of the bugs, operant conditioning was used to encourage the bugs to move opposite to their directional phototaxis. The learning association trials resulted in a statistically significant change in the behavior of the waterbugs. The results suggest that *B. flumineum* associates visual stimuli with a reward. These findings provide insight into the life history and potential for adaptive learning in *B. flumineum*.

## References

- Alcock, John. 2005. The development of behavior. *Animal Behavior: An Evolutionary Approach*. 55-98. Sinauer Associates. Sunderland, MA. ix+564 pp.
- Berthouex, P. M., and L. C. Brown. 1994. Assessing the Difference of Proportions. *Statistics for Environmental Engineers*. 115-12. Lewis. Boca Raton, FL. vii+335 pp.
- Bland, J. M., and D. G. Altman. 2000. The odds ratio. *BJM: British Medical Journal* 320:1468.
- Box, George E. P., William G. Hunter, and J. Stuart Hunter. 1978. *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building*. Wiley. New York, NY. xviii+653 pp.
- Brembs, B., 2003. Operant conditioning in invertebrates. *Current Opinion in Neurobiology* 13:710-717.
- Cammaerts, M. C., 2004. Operant conditioning in the ant *Myrmica sabuleti*. *Behavioural Processes* 67:417-425.
- Chatfield, C., and G. J. Goodhardt. 1970. The beta binomial model for consumer purchasing behavior. *Journal of the Royal Statistical Society* 19:240-250.
- Crowder, M. J. 1978. Beta-binomial Anova for Proportions. *Applied Statistics* 27:34-37.
- Daly, H. V., J. T. Doyen, and A. H. Purcell III. 1998. *Introduction to Insect Biology and Diversity*. Oxford University Press. New York, NY. vii+680 pp.
- Dukas, R. 2008. Evolutionary biology of insect learning. *Annual Review of Entomology* 53:145-160.

- Egas, M., and M. W. Sabelis. 2001. Adaptive learning of host preference in a herbivorous arthropod. *Ecology Letters* 4:190-195.
- Griffiths D. A., 1973. Maximum likelihood estimation for the beta-binomial distribution and an application to the household distribution of the total number of cases of a disease. *Biometrics* 29:637-648.
- Hebbsgaard, M. B., N. M. Andersen, and J. Damgaard. 2004. Phylogeny of the true water bugs (Nepomorpha: Hemiptera-Heteroptera) based on 16S and 28S rDNA and morphology. *Systematic Entomology* 29:488-508.
- Heffner, R. A., M. J. Butler, IV, and K. Reilly. 1996. Pseudoreplication revisited. *Ecology* 77:2558-2562.
- Hénaut, Y., C. Alauzet, D. Dargagnon, and M. Lambin. 1999. Visual learning in larval *Orius majusculus* a polyphagous predator. *Entomologia Experimentalis et Applicata* 90:103-107.
- Hénaut, Y., C. Alauzet, A. Ferran, and T. Williams. 2000. Effect of nymphal diet on adult predation behavior in *Orius majusculus* (Heteroptera: Anthrenidae). *Journal of Economic Entomology* 93:252-255.
- Honda, T., and Y. Kainoh. 1998. Age-related fecundity and learning ability of the egg larval parasitoid *Ascogaster reticulatus* Watanabe (Hymenoptera: Braconidae). *Biological Control* 13:177-181.
- Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54:187-211.
- Kazdin, A. E. 2001. *Behavior Modification in Applied Settings*. Waveland. Long Grove, IL. xiv+512 pp.

- Kelly, K. M., and M. I. Mote. 1990. Avoidance of monochromatic light by the cockroach *Periplaneta americana*. *Journal of Insect Physiology* 36:287-291.
- Kettlewell, H. B. D., and D. L. T. Conn. 1977. Further background-choice experiments on cryptic Lepidoptera. *Journal of Zoology* 181:371-376.
- Kight, S. L., and K. C. Kruse. 1992. Factors affecting the allocation of paternal care in waterbugs (*Belostoma flumineum* Say). *Behavioral Ecology and Sociobiology* 30:409-414.
- Kraus, W. F. 1989. Surface wave communication during courtship in the giant waterbug, *Abedus indentatus* (Heteroptera: Belostomatidae). *Journal of the Kansas Entomological Society* 62:316-328.
- Kruse, K. C. 1990. Male Backspace Availability in the Giant Water Bug (*Belostoma flumineum* Say). *Behavioral Ecology and Sociobiology* 26:281-89.
- Lee, J. C., and E. A. Bernays. 1990. Food tastes and toxic effects: associative learning by the polyphagous grasshopper *Schistocerca americana* (Drury) (Orthoptera: Acrididae). *Animal Behaviour* 39:163-173.
- Lord, F. M. 1965. A true-story theory, with applications. *Psychometrika* 30:239-270.
- Madden, L. V., G. Hughes, and M. A. Ellis. 1995. Spatial heterogeneity of the incidence of grape downy mildew. *Phytopathology* 85:269-275.
- Mery, F., and T. J. Kawecki. 2003. A fitness cost of learning ability in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences* 270:2465-2469.
- Mery, F., and T.J. Kawecki. 2004. An operating cost of learning in *Drosophila melanogaster*. *Animal Behaviour* 68:589-598.



- Moore, D. S., G. P. McCabe, and B. A. Craig. 2012. Sampling Distributions. *Introduction to the Practice of Statistics*. 297-339. W.H. Freeman and Company. New York, NY. xxxi+694 pp.
- Orlosk, J. L., J. M. Walker, A. L. Morrison, and J. Atema. 2011. Conditioning the crab *Carcinus maenas* against instinctive light avoidance. *Marine and Freshwater Behaviour and Physiology* 44:375-381.
- Padaj, D. R., and A. C. Lewis. 1993. *Insect Learning: Ecology and Evolutionary Perspectives*. Chapman and Hall. New York, NY. xiii+398 pp.
- Polhemus, J. T., and D.A. Polhemus. 2008. Global diversity of true bugs (Heteroptera; Insecta) in freshwater. *Hydrobiologia* 595:379-391.
- Punzo, F. 1984. Recent advances in behavioral plasticity in insects and decapods crustaceans. *The Florida Entomologist* 68:89-104.
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. <<http://www.R-project.org/>>.
- Schuh, R. T., and J. A. Slater. 1995. Belostomatidae. *True Bugs of the World: Hemiptera, Heteroptera: Classification and Natural History*. 111-114. Cornell University Press. Ithaca, NY. xii+337 pp.
- Severin, H. P., and H. C. Severin. 1911. Habits of *Belostoma* (=Zaitha) *flumineum* Say and *Nepa Apiculata* Uhler, with observations on other closely related aquatic Hemiptera. *Journal of the New York Entomological Society* 19:99-108.

- Smith, B.H. 1993. Merging mechanism and adaptation: an ethological approach to learning and generalization. *In* Papaj D. R. and A. C. Lewis (eds). *Insect Learning: Ecological and Evolutionary Perspectives*. 126-157. Chapman & Hall. New York, NY. xiii+398 pp.
- Smith, R. L. 1976. Brooding behavior of a male waterbug *Belostoma flumineum* (Hemiptera: Belostomatidae). *Journal of the Entomological Society* 39:333-343.
- Smith, R. L. 1997. Evolution of paternal care in the giant waterbugs (Heteroptera: Belostomatidae). *In* Choe, J. C. and Bernard J. Crespi (eds). *The Evolution of Social Behavior in Insects and Arachnids*. 116-149. Cambridge University Press. Cambridge, UK. xiii+541 pp.
- Smith, R. L. 1979. Paternity assurance and altered roles in the mating behaviour of a giant waterbug, *Abedus herberti* (Heteroptera: Belostomatidae). *Animal Behaviour* 27:716-725.
- Staddon, J. E. R. 1983. *Adaptive Behavior and Learning*. Cambridge University Press. Cambridge, UK. xi+561 pp.
- Stephens, D. W. 1993. Learning and behavioral ecology: incomplete information and environmental predictability. *In* Papaj, D. R. and A.C. Lewis (eds). *Insect Learning: Ecology and Evolutionary Perspectives*. 195-218. Chapman & Hall. New York, NY. xiii+398 pp.
- Tallamy, D. W. 2001. Evolution of exclusive parental care in arthropods. *Annual Review of Arthropods* 46:139-165.
- Thorpe, W. H. 1943. Types of learning in insects and other arthropods. *British Journal of Psychology* 33:220-34.

- Vowles, D. M. 1965. Maze learning and visual discrimination in the wood ant (*Formica rufa*). *British Journal of Psychology* 56:15-31.
- Wade, C. and C. Tavris. 2005. Operant Conditioning. *Psychology*. 240-250. Pearson Prentice Hall. Upper Saddle River, NJ. xxviii+766 pp.
- Weiss, M. R. 1995. Associative colour learning in a nymphalid butterfly. *Ecological Entomology* 20:298-301.
- Weiss, M. R., 1997. Innate colour preferences and flexible colour learning in the pipevine swallowtail. *Animal Behaviour* 53:1043-1052.
- Welsh, J. H., 1934. The caudal photoreceptor and responses of the crayfish to light. *Journal of Cellular and Comparative Physiology* 4:379-388.
- Wilcox, R. R. 1981. The beta-binomial model and its extensions. *Journal of Educational Statistics* 6:3-32.
- Williams, D. A. 1975. The analysis of binary responses from toxicological experiments involving reproduction and tetratogenicity. *Biometrics* 31:949-952.
- Zhang, Z.-Q., 1992. Phototactic and geotactic responses in *Allothronbium pulvinum* larvae (Acari: Trombidiidae ). *Experimental and Applies Acarology* 15:41-47.

## Appendix

### Covariance Calculation

Covariance is the measure of how two separate variables change together (Moore *et al.*, 2012). As part of the statistical analysis described in the Results section, the data were also analyzed to determine if there was an interaction between the two primary variables (*i.e.*, room orientation bias and learning). Another beta binomial regression was run with 3 parameters, room orientation bias, learning, and the product of learning and room orientation bias. The coefficient for the product of these variables was 0.2129 and it had a p-value of 0.4311. The p-value was greater than 0.05, which indicates that the covariance between the two variables was not statistically significant. This suggests that the room orientation bias is constant throughout the experiments and does not affect the response to the operant conditioning.

## Tables and Figures

		Trial	
		Control	Learning
Southwest Corner Color	Black	LCL: 21%	LCL: 29%
		<b>25%</b>	<b>34%</b>
	White	UCL: 29%	UCL: 40%
		LCL: 26%	LCL: 35%
		<b>31%</b>	<b>41%</b>
		UCL: 35%	UCL: 47%

Table 1: Table of probabilities. Probability that the bugs will choose the white substrate under the 4 different conditions. LCL is the Lower Confidence Limit and the UCL is the Upper Confidence Limit. These are the 95% confidence limits on the probabilities.

		Trial	
		Control	Learning
Southwest Corner Color	Black	Probability of a control bug going to the white substrate when the southwest corner is black	Probability of a learning bug going to the white substrate when the southwest corner is black
	White	Probability of a control bug going to the white substrate when the southwest corner is white	Probability of a learning bug going to the white substrate when the southwest corner is white

Table 2: Explanation of the probabilities presented in Table 1.

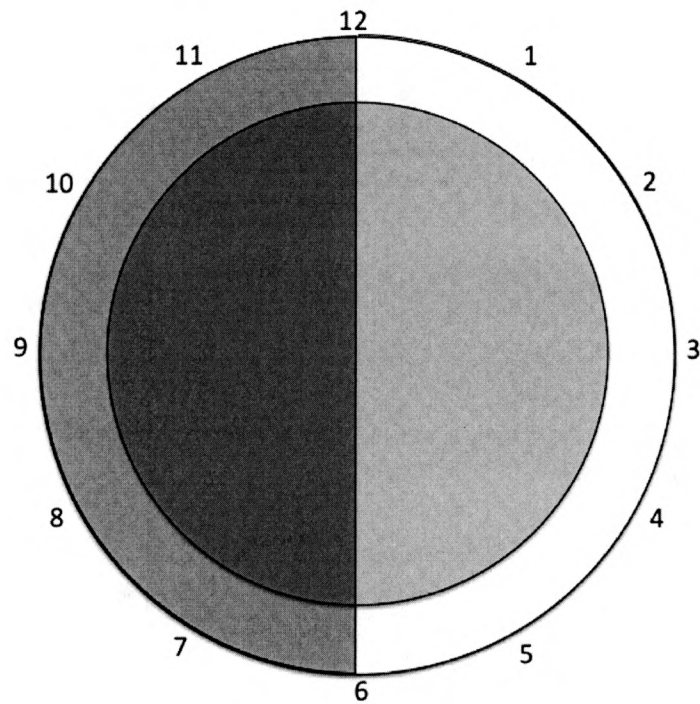


Figure 1: Overhead view of the basin used in Experiment 1. The basin was labeled with numbers 1-12 and half of it was shaded.

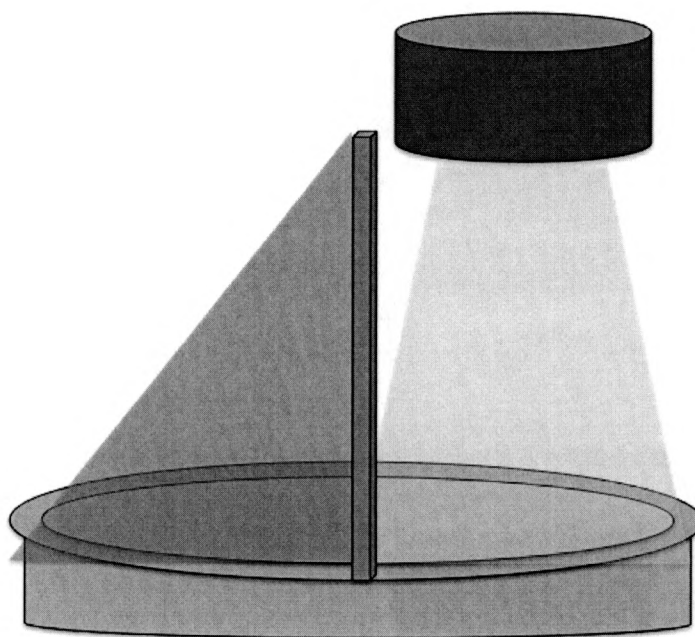


Figure 2: Side view of basin set-up used in Experiment 1.

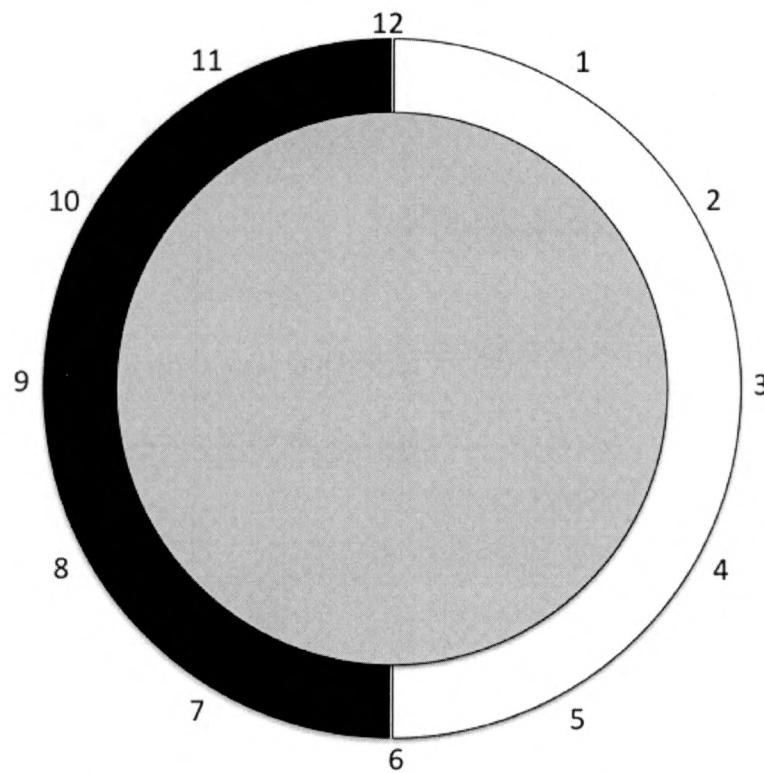


Figure 3: Overhead view of basin in Experiments 2 and 3. The basin was labeled with the numbers 1-12 and black and white paper was placed around the outside edge.



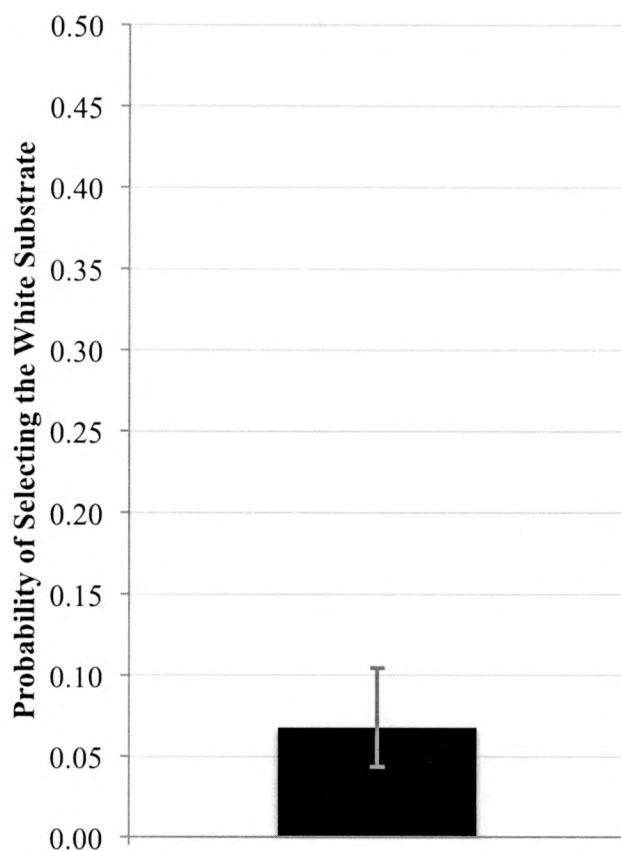


Figure 4: Probability of the waterbug selecting the white side of the basin in Experiment

1. The error bar indicates the 95% confidence limits on the probability.

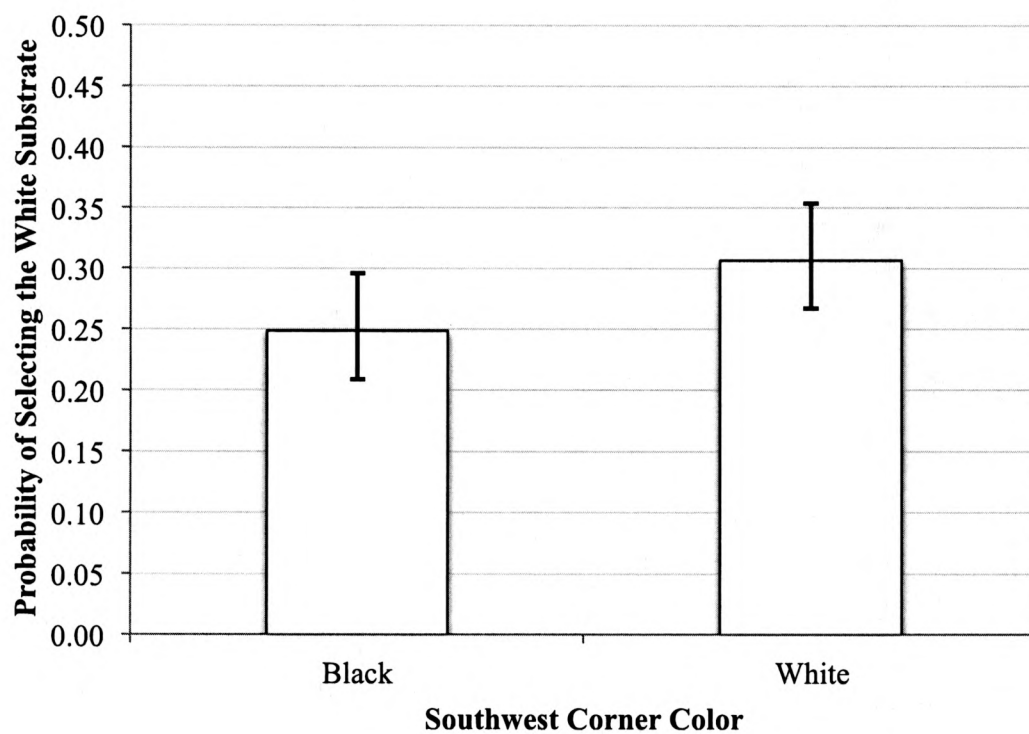


Figure 5: Probability of the waterbug selecting the white side of the basin in Experiment

2. The error bars indicate the 95% confidence limits on the probabilities.

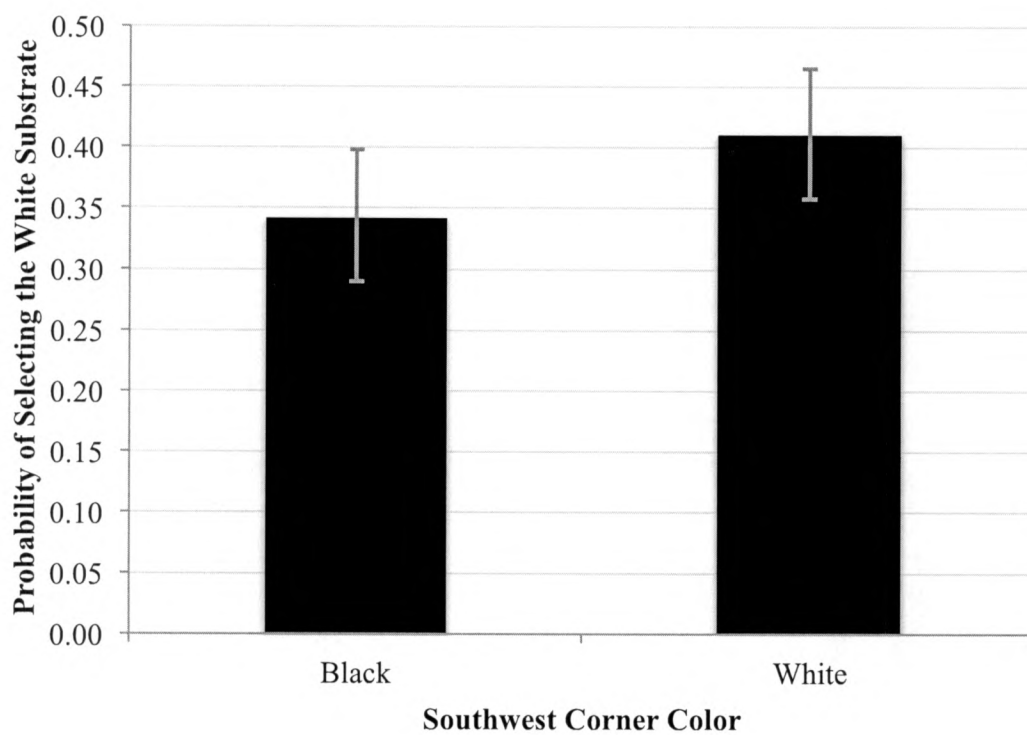


Figure 6: Probability of the waterbug selecting the white side of the basin in Experiment

3. The error bars indicate the 95% confidence limits on the probabilities.

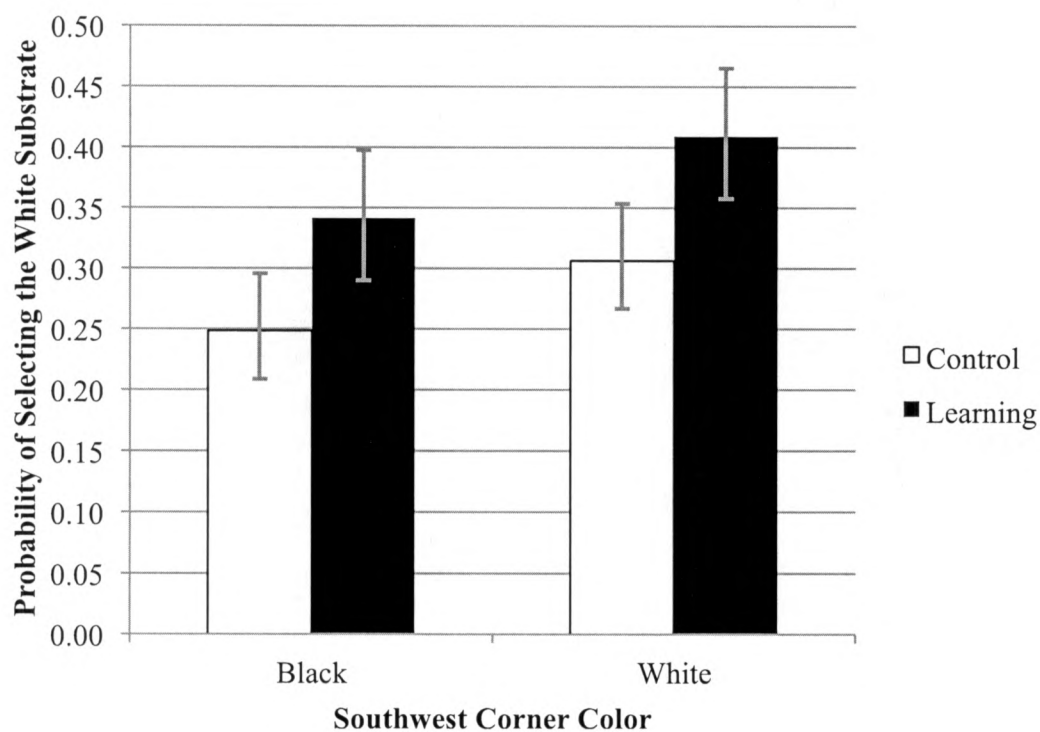


Figure 7: Probability of the waterbugs selecting the white side of the basin for Experiment 2 (control) and Experiment 3 (learning) under the 4 different conditions. The error bars indicate the 95% confidence limits on the probabilities.